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Can the excretion of metabolites by bacteria be manipulated?

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1. SUMMARY

Bacteria can release metabolites into the environment by various mechanisms. Excretion may occur by passive diffusion or by the reversal of the uptake process when the internal concentration of the metabolite exceeds the thermodynamic equilibrium level. In other cases, solutes are excreted against the concentration gradient by special extrusion systems. Their mode of energy coupling is different to that of the well-studied group of uptake systems. A thorough understanding of the transport processes will help to improve the excretion of metabolites of commercial interest, allow a more efficient production of metabolites in bulk quantities, and permit their exploitation to establish new markets.

2. INTRODUCTION

For growth and survival, bacteria have to take up different compounds from the environment.

These compounds (solutes) can vary from carbon, energy, nitrogen, phosphorus and sulphur sources to precursors for the biosynthesis of macromolecules. All these solutes have to pass the cell envelope of which the cytoplasmic membrane forms a highly selective barrier. Metabolism of solutes leads to the formation of intermediary metabolites and endproducts. These products of metabolism (metabolites) can be excreted into the environment if they can cross the membrane by passive diffusion, if the concentration of the metabolites in the cytoplasm increases beyond the thermodynamic equilibrium level or if specific excretion systems are active. The excretion of many metabolites by bacteria is of commercial interest and large scale fermentation processes are used for the production of bulk quantities of such metabolites. Examples are the production of the amino acids lysine and glutamate by *Corynebacterium glutamicum*. Commercial interest has led to attempts to enhance the rate and yield of metabolite production. In some cases, novel bacterial species or strains have been successfully applied for the enhanced production of a metabolite. The metabolic pathways which lead to the production of a metabolite are usually well-analysed and medium conditions have been optimized to establish commercial production.

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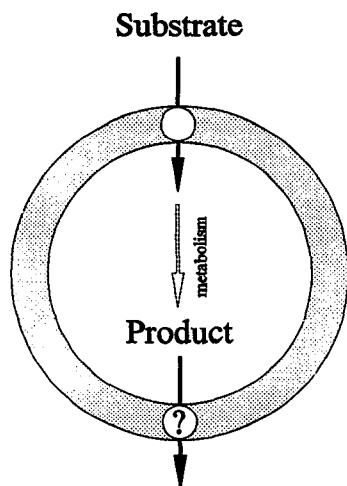


Fig. 1. Schematic representation of the conversion of a substrate to a product by bacteria.

The conversion of substrates to products involves several different metabolic activities (Fig. 1). First the substrate has to be translocated from the environment to the cytoplasm. Subsequently, the substrate has to be converted into products by one or more enzymatic reaction(s). Finally, the product has to be translocated across the cytoplasmic membrane to the external medium. Manipulation of the production of metabolites has focused mainly on metabolism. This includes the use of specific growth conditions, selection of mutants with key enzymes having altered feedback inhibition, and the control of enzyme levels by genetic techniques. Attempts have also been made to direct the metabolite flow by increasing the rate of product excretion through manipulation of the physical properties of the cytoplasmic membrane.

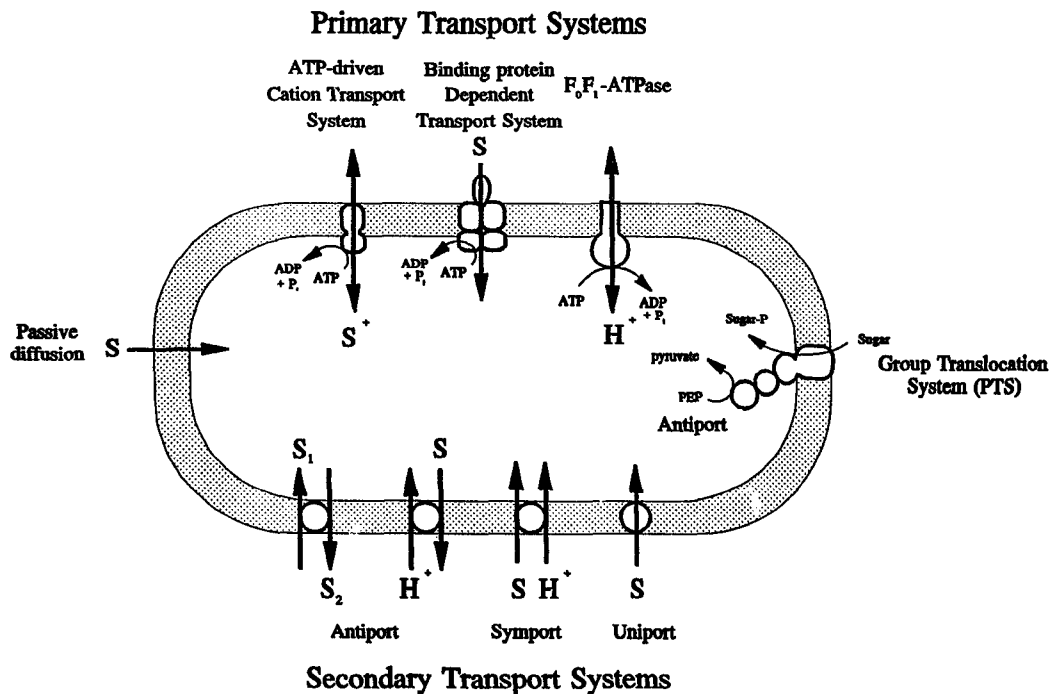


Fig. 2. Mechanisms of solute transport in bacteria. Solutes and protons are indicated by S (S_1 , S_2) and H^+ , respectively. Secondary transport systems may use Na^+ instead of H^+ .

This review describes the different mechanisms which can be involved in the translocation of solutes from the cytoplasm to the external medium. Strategies are discussed as how to manipulate these mechanisms and optimize product excretion.

3. MECHANISMS OF SOLUTE TRANSPORT

Before discussing the different mechanisms involved in excretion processes, the mechanisms which can play a role in the translocation of solutes across the cytoplasmic membrane of bacteria will be described (Fig. 2).

3.1. Passive transport

The most simple mechanism is passive transport in which solutes cross the membrane without the involvement of a specific transport protein. All other transport mechanisms require specific integral membrane proteins. The driving force for passive transport of a neutral solute is supplied by the chemical gradient of the solute.

3.2. Primary transport systems

Primary transport systems convert chemical or light energy into electrochemical energy. Systems identified within this group are the cytochrome-linked electron transfer systems such as the respiratory chains in aerobic bacteria and the cyclic electron transfer systems in phototropic bacteria [1,2]. In most bacteria, the activities of these systems are coupled to the translocation of protons from the cytoplasm to the external media. Proton translocation leads to the formation of an electrochemical gradient of protons which is composed of a transmembrane electrical potential ($\Delta\psi$, inside negative versus outside) and pH gradient (ΔpH , inside alkaline versus outside). Both the $\Delta\psi$ and ΔpH exert a force on the protons, the protonmotive force (Δp), which drives the protons from the outside to the inside. In equation:

$$\Delta p = \frac{\Delta\bar{\mu}_{\text{H}^+}}{F} = \Delta\psi - Z\Delta\text{pH} \quad (\text{mV}) \quad (1)$$

in which Z equals RT/F . R , T and F represent the gas constant ($8.31 \text{ J mol}^{-1} \text{ K}^{-1}$), absolute temperature (K) and Faraday constant ($96\,500 \text{ Coulomb mol}^{-1}$), respectively.

In some bacteria, such as *Vibrio alginolyticus*, electron transfer is coupled to the translocation of Na^+ ions [3]. The activity of the electron transfer system then leads to the generation of a chemical gradient of Na^+ ions in addition to a $\Delta\psi$, i.e. the electrochemical gradient of Na^+ ions:

$$\frac{\Delta\bar{\mu}_{\text{Na}^+}}{F} = \Delta\psi + \frac{\Delta\bar{\mu}_{\text{Na}^+}}{F} \quad (\text{mV}) \quad (2)$$

A simple primary ion-translocating system is bacteriorhodopsin. Light, absorbed by the retinal-containing chromophore, provides the energy for uphill transport of protons [4]. Other primary transport systems are the membrane-bound ATPases. ATP-hydrolysis by these systems results in the translocation of solutes or ions depending on the specificity of the system. The F_0F_1 -ATPase couples hydrolysis of ATP to the translocation of protons across the membrane [5]. The activity of this system will lead to the generation of a Δp . This only occurs when the phosphorylation potential, ΔG_p , exceeds $n\Delta p$ (n is the number of protons translocated per ATP hydrolysed). Since both have a negative value this means if $\Delta G_p - n\Delta p < 0$, ΔG_p equals:

$$\Delta G_p = \Delta G_p^0 - \frac{2.3 RT}{F} \log \left(\frac{[\text{ATP}]}{[\text{ADP}] \cdot [\text{P}_i]} \right) \quad (\text{mV}) \quad (3)$$

in which ΔG_p^0 is the standard free energy of ATP hydrolysis or synthesis. The reversibility of the F_0F_1 -ATPase permits the synthesis of ATP at the expense of Δp when $\Delta G_p - n\Delta p > 0$. Some F_0F_1 -ATPases translocate Na^+ ions instead of protons [6].

Other ATPases have been found to mediate the uptake of K^+ and Mg^{2+} ions or the export of a variety of toxic ions, including Ca^{2+} , Cd^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} , Hg^{2+} , CrO_4^{2-} , Cu^{2+} and oxyanions (arsenate, arsenite, and antimonite) [7–9]. The marked diversity of the group of transport ATPases is further demonstrated by the binding-protein-dependent transport systems pre-

sent in Gram-negative bacteria [10]. These systems are involved in the uptake of a large variety of substrates such as amino acids, (oligo-)peptides, sugars, vitamins, anions (sulfate, phosphate, molybdate) and cations (Fe^{3+} , Cu^{2+}). These complex multisubunit membrane proteins are typically composed of a periplasmic solute-binding protein and three membrane-bound components. The free energy of the hydrolysis of ATP is used to concentrate solutes inside the cell against very large gradients (up to 10^5 -fold). Representatives of this group have recently been found in Gram-positive bacteria; the binding protein in these organisms is bound to the membrane surface by a lipid anchor [11–13]. Systems involved in the export of macromolecules like proteins and polysaccharides (see Section 4) show sequence similarity with components of the family of periplasmic permeases. The ABC (ATP Binding Cassette) superfamily of transport ATPases [10] includes many prokaryotic and eukaryotic transport systems with similar global structures.

3.3. Secondary transport systems

Secondary transport systems convert one form of electrochemical energy into another form. The energy for translocation of a solute via these systems is supplied by (electro-)chemical gradients and is converted into an (electro-)chemical gradient of the solute. Three different types of secondary transport systems can be distinguished: *uniport* systems which translocate a single solute across the membrane; *symport* systems which mediate coupled translocation of two solutes in the same direction, and *antiport* systems which couple the translocation of one solute in one direction to the translocation of another solute in the opposite direction. Uniport systems are less common. A special form of uniport is the glycerol facilitator of *Escherichia coli* which mediates downhill influx of glycerol. Most bacterial transport systems belong to the group of solute symport systems [14]. For instance, amino acids are often translocated by symport systems. The symported ions are usually protons, but in an increasing number of cases Na^+ is found to be the co-substrate [15]. Two different types of antiport systems are operating in bacteria. One type func-

tions in the excretion of unwanted cations such as Na^+ and Ca^{2+} at the expense of proton uptake. The second type of antiport systems mediate the coupled exchange of a substrate (precursor) of a metabolic pathway with one of the products of this pathway. Examples of these systems will be covered in this review.

3.4. Group translocation systems

Group translocation systems couple the translocation of a solute to the chemical modification of the solute resulting in the release of a modified solute at the other side of the membrane (Fig. 2). The only group-translocation systems found up to date in bacteria are the phosphoenol-pyruvate-dependent sugar translocation systems (PTS) [16]. These systems are involved in the uptake of sugars, and consist of membrane-bound and soluble proteins. During the translocation process the sugar is phosphorylated and released at the inner face of the cytoplasmic membrane.

4. EXCRETION MECHANISMS

In principle, all the transport systems described above can function in the opposite direction and thus can play a role in the excretion of certain metabolites. Among the primary transport systems some ATP-driven transport systems are designed to excrete solutes, i.e. toxic inorganic ions [8,9], capsular polysaccharides [17,18], cyclic β -(1 \rightarrow 2) glucans [19,20], antibiotics such as erythromycin [21] and a variety of extracellular proteins (haemolysin, metalloprotease, leukotoxin and adenylate cyclase) [10,22]. Most of the transport ATPases, however, are directed to accumulate solutes at the expense of phosphate-bond energy. With the exception of the F_0F_1 -ATPases (see above), energetic conditions are seldom favourable for the reversed action, e.g. ATP synthesis activity. Also group translocation systems essentially only function to transport sugars into the cell, although efflux of free sugar by the integral membrane domain, enzyme II of PTS has been reported [23]. The main mechanisms for metabolite excretion are therefore restricted to

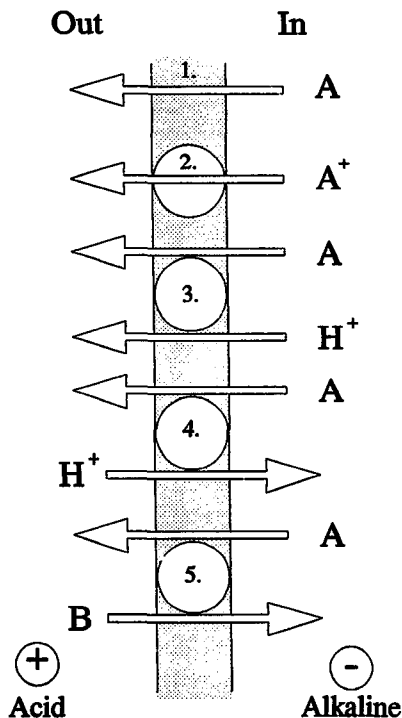


Fig. 3. Excretion mechanisms in bacteria. 1. Passive translocation of a neutral solute A. 2. Excretion of a positively charged solute A^+ via a uniport mechanism. 3. Excretion of a neutral solute A via a solute/proton symport mechanism. 4. Excretion of a neutral solute A via a solute/proton antiport mechanism. 5. Exchange of neutral solutes A and B via an antiport mechanism.

passive or secondary transport processes. Before describing these excretion systems in more detail the forces which play a role in the translocation processes have to be considered (Fig. 3).

Passive translocation of a neutral solute A across the membrane (Fig. 3, example 1) is driven only by the chemical gradient of A:

$$\frac{\Delta\bar{\mu}_A}{F} = \frac{2.3 RT}{F} \log \left(\frac{[A_{in}]}{[A_{out}]} \right) \quad (\text{mV}) \quad (4)$$

The process will continue until the external concentration equals the internal concentration, i.e. $\Delta\bar{\mu}_A/F = 0$. The second example shows the excretion process of a positively charged solute A^+

by a uniport system. In this process $\Delta\bar{\mu}_{A^+}/F$ functions as a driving force for excretion. Since solute A^+ is positively charged, $\Delta\psi$ (inside negative) exerts an inwardly directed, counteracting force such that the total driving force on A^+ equals:

$$\frac{\Delta\bar{\mu}_{A^+}}{F} + \Delta\psi \quad (\text{mV}) \quad (5)$$

If the driving force is positive, A^+ will be driven to the outside ($[A^+]_{in} < [A^+]_{out}$), if the sum is zero, the outwardly directed force of the gradient of A^+ is compensated by the inwardly directed force supplied by $\Delta\psi$ ($[A^+]_{in} > [A^+]_{out}$); and if the driving force is negative the net driving force will be directed to the inside and no net excretion of A will occur. The third example shows an excretion process via a solute/proton symport mechanism. A neutral solute A is excreted in symport with one proton and the electrochemical gradients of both solutes supply the driving forces for this translocation process. These forces are the chemical gradient of A ($\Delta\bar{\mu}_A/F$) and the electrochemical gradient of protons ($\Delta\bar{\mu}_{H^+}/F$).

$$\frac{\Delta\bar{\mu}_A + \Delta\bar{\mu}_{H^+}}{F} \quad (\text{mV}) \quad (6)$$

The driving force on the protons is directed inward and opposite to the direction of excretion. Solute excretion via this process will only occur if the net driving force is positive, i.e. conditions at which the driving force supplied by the solute gradient exceeds the driving force supplied by Δp . Solute efflux will proceed until the net driving force is zero (See eqn. 6.). For charged solutes, the equations given in Table 1 can be applied.

The fourth example (Fig. 3) concerns an antiport system in which the excretion of solute A is coupled to the uptake of a proton. Again the driving forces are supplied by the (electro-)chemical gradients of the transported solutes as defined in eqn. 7.

$$\frac{\Delta\bar{\mu}_A - \Delta\bar{\mu}_{H^+}}{F} \quad (\text{mV}) \quad (7)$$

Since the inward flow of protons is coupled to the outward movement of solute A, the force on the

protons is directed towards the excretion of the solute. Thus, Δp directly contributes to the excretion process. This system is therefore optimally suitable for a metabolite excretion mechanism. The final example (Fig. 3, example 5) again shows an antiport system, but now concerns a system in which the excretion of solute A is coupled to the uptake of solute B. If no net charge is translocated, the driving force of this process is supplied by the chemical gradient of both solutes and equals:

$$\frac{\Delta\bar{\mu}_A - \Delta\bar{\mu}_B}{F} \quad (\text{mV}) \quad (8)$$

Table 1 gives the general equations for the driving force for excretion according to the mechanisms discussed when either the charge of solute A and B, or the number of cotransported protons is varied. In all these examples and other systems which can be conceived, excretion of solute will occur if the net driving force is positive. Excretion will continue until the net driving force is zero. These systems will work in the uptake mode when the net driving force is negative, allowing the translocation of solutes into the cell. A proper understanding of the forces involved in excretion of a metabolite therefore requires detailed knowledge about the mechanism of excretion. This includes the net charge and the number of protons (or other ions) translocated as well as quantitative information on the magnitude of the steady-state solute, electrical and proton gradients.

5. SPECIFIC EXCRETION PROCESSES

5.1. Passive diffusion

Fick's first law of diffusion represents the simplest possible model for the passive flux (J_{mem}) of an uncharged solute across the membrane (eqn. 9.) (See references [24] and [25]). This model assumes that (i) the diffusion rate across the membrane is slow compared to the rate of diffusion in the aqueous phase, (ii) the membrane functions as a homogeneous layer; and (iii) in steady state the concentration of a solute just in the membrane is in equilibrium with the concentration of the solute in the aqueous phase and in the centre of the membrane.

$$J_{\text{mem}} = \frac{K \cdot D}{L} A (c_{\text{in}}^{\text{aq}} - c_{\text{out}}^{\text{aq}}) \\ = PA (c_{\text{in}}^{\text{aq}} - c_{\text{out}}^{\text{aq}}) \quad (\text{in } \text{M cm}^3 \text{ s}^{-1}) \quad (9)$$

in which the thickness (in cm) and surface area (in cm^2) of the membrane are represented by L and A , respectively. D is the diffusion coefficient (in cm^2/s), P is the permeability coefficient (in cm/s) and K the partition coefficient defined as:

$$K = \frac{c_{\text{in}}^{\text{mem}}}{c_{\text{in}}^{\text{aq}}} = \frac{c_{\text{out}}^{\text{mem}}}{c_{\text{out}}^{\text{aq}}} \quad (10)$$

c signifies the steady-state concentrations of solute c just in and outside the membrane at either the inner and outer face of the membrane. Small solutes permeate across the membrane by disso-

Table 1

General equations for the driving forces of solute excretion mechanisms

Mechanism	Solutes		Driving force
	Outside	Inside	
Uniport		A^m	$\Delta\bar{\mu}_{A^m}/F + m \cdot \Delta\psi$
Symport		nH^+, A^m	$\Delta\bar{\mu}_{A^m}/F + (n+m) \cdot \Delta\psi - n \cdot Z\Delta pH$
Antiport	nH^+	A^m	$\Delta\bar{\mu}_{A^m}/F - (n-m) \cdot \Delta\psi + n \cdot Z\Delta pH$
	B^k	A^m	$\Delta\bar{\mu}_{A^m}/F - \Delta\bar{\mu}_{B^k}/F - (k-m) \cdot \Delta\psi$
	nH^+, B^k	qH^+, A^m	$\Delta\bar{\mu}_{A^m}/F - \Delta\bar{\mu}_{B^k}/F - (k+n-m-q) \cdot \Delta\psi + (n-q) \cdot Z\Delta pH$

The variables k , m , n and q are integers. The sign of k and m is either positive or negative depending on the charge of solute A and B, respectively. n and q represent the number of protons co-transported. The sign of n and q is positive.

lution and diffusion in the lipid bilayer regions of the membrane.

Hydrophobic metabolic endproducts such as butanol, acetone and organic acids rapidly partition into the lipid membrane [26,27]. At high concentrations, these compounds may disrupt the membrane structure and inhibit bacterial growth [28–30]. Organic acids such as acetate and lactate are excreted passively in some bacteria and carrier-mediated in others [31]. At low pH the concentration of the undissociated organic acid can become high. The undissociated form of some of these organic acids readily dissolves into the lipid membrane [27]. Partitioning of these metabolites into the membrane (See eqn. 10.) depends on the lipophilicity of the acid. The concentration in the membrane is directly related to the concentration in the cytoplasm and the external medium. The rate of passive diffusion is dependent on the transmembrane concentration gradient (eqn. 9.). By keeping the external concentration low, thereby maximizing the concentration gradient, efflux via passive diffusion can be increased. The degree of dissociation of these acids will directly depend on the internal pH. However, most bacteria keep their internal pH around neutrality and many organic acids are present in the cytoplasm mainly in their dissociated form. At low medium pH, the concentration of the undissociated organic acids in the cytoplasmic membrane can become too high and exert an uncoupling action which dissipates the Δp and leads to dissipation of metabolic energy. Such effects have for instance been observed during the production of acetate and butyrate by *Clostridia* species [32–34]. Some bacteria can avoid the inhibitory effects of the organic acids at low pH by directing their metabolism towards the production of organic solvents like butanol and acetone [35,36].

Passive fluxes are also determined by the physical properties of the phospholipid bilayer, such as acyl chain composition (G. In't Veld, A.J.M. Driessen and W.N. Konings, unpublished results) and viscosity (see eqn. 9). Coryneform bacteria are widely used for the production of glutamate and lysine [37]. Although these amino acid production processes are of considerable industrial importance, the mechanisms of excretion remain

obscure. Various mechanisms have been proposed [37–42]. Until now, the most widely accepted mechanism for the excretion of these metabolites has been passive diffusion across the cytoplasmic membrane. The observations that excretion of glutamate by *C. glutamicum* [37,43,44] and 5'-inosinic acid [45] and NAD [46] by *Brevibacterium ammoniagenes* can be induced by various treatments which cause alterations of the bacterial membrane would be consistent with a passive diffusion mechanism. These conditions are: biotin limitation, treatment with detergents, manganese depletion, addition of penicillin and fermentation using oleic acid or glycerol auxotrophs. However, at present excretion of these amino acids is thought to occur via specific efflux transport systems (see below).

Passive diffusion of amino acids across bacterial cell membranes has extensively been studied by Driessen et al. [47] in *Lactococcus lactis*. The first order rate constant of passive diffusion ($K \cdot D$ in eqn. 9.) of several amino acids was determined in membrane vesicles of *L. lactis* fused with cytochrome *c* oxidase-containing liposomes [48]. The steady-state level of amino acid accumulation in these hybrid membranes decreased with increasing hydrophobicity of the amino acid side chain. First-order rate constants for amino acid efflux were estimated from the initial rate of amino acid uptake and the steady-state accumulation level, and corrected for the contribution of carrier-mediated efflux. Since the membrane thickness in this experiment was equal for all amino acids tested, $K \cdot D$ is directly proportional to P . The hydrophobicity of the side chain [49] of the amino acids and $\ln(K \cdot D)$ are correlated linearly (Fig. 4). Such a behaviour is as predicted for a simple passive diffusion process, and consistent with observations on the permeability of lipid vesicles for amino acids [50,51].

In vivo, diffusion of amino acids across the cytoplasmic membrane can be significant. *L. lactis* relies on passive diffusion as transport mechanism to satisfy the demand for proline. This organism is a proline prototroph and lacks an uptake system for proline. During growth in milk the proline requirement is met by the uptake of proline-containing peptides. Proline is released

within the cell as the peptides are hydrolysed by intracellular peptidases [52]. Growth on defined media containing free amino acids as the sole nitrogen source is only possible when proline is present at millimolar concentrations. The rate of proline uptake is a linear function of the external proline concentration (Fig. 5). Passive fluxes of amino acids across the cytoplasmic membranes have also been observed in *E. coli* [53–56], *Salmonella typhimurium* [57,58] and many other bacteria [59–61].

For the large scale production of amino acids, we must realize that excretion of amino acids by passive efflux can be counteracted by active uptake of these amino acids. A general strategy for

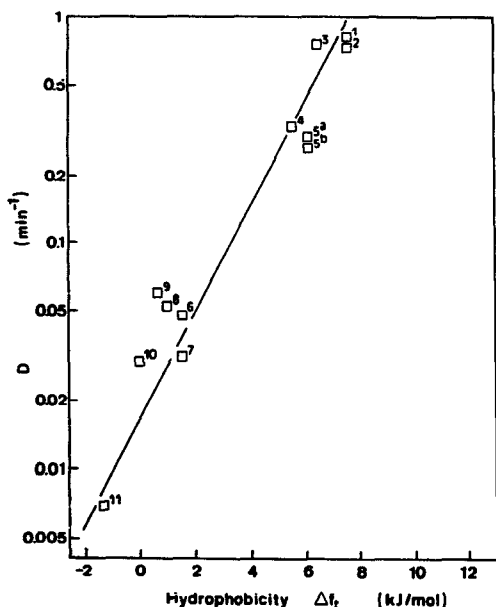


Fig. 4. Relation between the relative hydrophobicity of the side chain of amino acids and the first order rate constant of passive diffusion of the amino acids in membrane vesicles of *L. lactis* ssp. *cremoris* Wg₂ fused with cytochrome *c* oxidase proteoliposomes. 1, L-leucine; 2, L-isoleucine; 3, L-valine; 4, L-methionine; 5^a, L-lysine (H⁺-symport); 5^b, L-lysine (uniport); 6, L-histidine; 7, L-alanine; 8, L-threonine; 9, L-proline; 10, glycine; and 11, L-serine. Reproduced from reference [47] with permission.

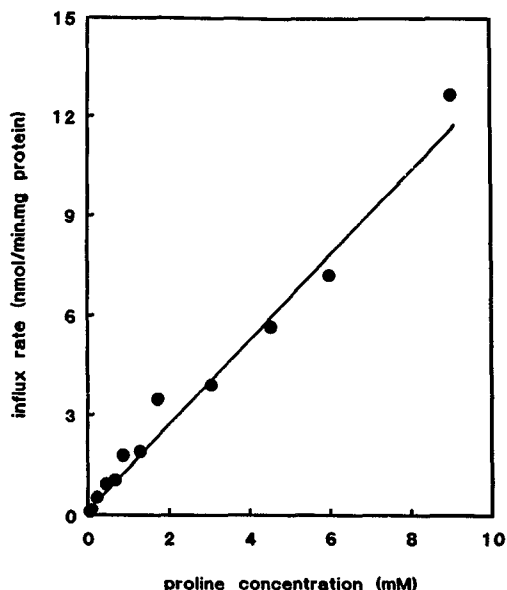


Fig. 5. Kinetics of proline influx into intact cells of *L. lactis*; ssp. *lactis* ML₃. Reproduced from reference [52] with permission.

enhancing the excretion of amino acids or other products is the elimination of the active transport systems for these compounds. This is clearly exemplified by the studies of Rancourt et al. [56] on the excretion of proline by *E. coli*. Proline excretion is not caused by uncontrolled biosynthesis of proline, but results rather from a process that occurs when catabolism or transport are defective. These results demonstrate that, in biological membranes, considerable leaks of hydrophobic solutes can occur. For hydrophilic solutes the passive flux across the membrane will be insignificant. This is advantageous for the organism as it would otherwise rapidly lose its metabolites.

5.2. Ion/solute symport

The direction of a secondary transport system depends on the polarity of the driving force and thus is determined by the electrochemical gradients of the solutes transported (eqn. 6.). In case of a solute/proton symport system the driving force exerted on the protons ($\Delta\mu_{\text{H}^+}/F$) is usually

directed from the outside to the inside. For solutes which are metabolized within the cell, the chemical gradient of the solute ($\Delta\bar{\mu}_A/F$) is also directed to the inside. The system will therefore operate in the uptake mode. A different situation might be encountered when a metabolic intermediate or endproduct of metabolism is produced in the cell. The concentration of the product in the cell will increase and rapidly exceed the external concentration. If the transmembrane gradient of the product exceeds the electrochemical gradient of protons the product will be driven from the cytoplasm to the external medium (eqn. 11.).

$$\frac{\Delta\bar{\mu}_A + \Delta\bar{\mu}_{H^+}}{F} > 0 \quad (\text{mV}) \quad (11)$$

This situation occurs for metabolite endproducts in fermentative bacteria. During the fermentation process large quantities of endproducts are produced in the cell and these products have to leave the cell, in some cases via solute/proton symport systems.

For instance, during glucose or lactose fermentation in *E. coli* [62] and *L. lactis* [63], the lactate produced is excreted in symport with protons. The excretion process has been studied in vivo [63–65] and in membrane vesicles [62,66] within the pH range 5 to 8. The apparent pK of lactate is 3.08, and at high intracellular pH values essentially all lactate is in the anionic form. At high pH values the excretion process was found to be electrogenic, indicating that more than one proton is translocated per lactate anion. At low pH values lactate excretion is an electroneutral process and only one proton is translocated per lactate anion. These observations indicate that at high pH values lactate is excreted by a proton symport system with two or more protons [67]. At low pH values, lactate excretion can occur by the same transport system but with a proton lactate stoichiometry of one and by passive diffusion of the undissociated lactic acid. At low internal pH, the lactate/proton symport system could become inactivated [68] and passive diffusion is then the only available excretion pathway.

Excretion of lactate in symport with protons leads to the generation of a Δp . This process can therefore contribute to the metabolic energy pro-

duction during the fermentation process. These considerations have led to the 'energy recycling model' which postulates that excretion of a metabolic endproduct via an ion symport system may lead to the generation of metabolic energy [69]. In recent years evidence has been presented for H^+ -linked carrier-mediated excretion of lactate in *L. lactis* [63,66], *E. coli* [62] and *Enterococcus faecalis* [70,71], and Na^+ -linked excretion of succinate in *Selenomonas ruminantium* [72]. Carrier-mediated secretion of acetate has been suggested in *Syntrophomonas wolfei* [73], *Desulfovibrio desulfuricans* [73], several other sulfate reducers [74,75], *Acinetobacter woodii* [76] and some methanogens [77].

The rate of carrier-mediated excretion of metabolites via ion/solute symport systems depends on the magnitude of the driving force. Excretion rates can be increased by decreasing the Δp or by increasing the chemical gradient of the solute. The latter can be realized when the external concentration is lowered by dilution, dialysis, or by a chemical or enzymatic conversion of the endproduct. Another procedure relies on the acceleration of metabolism which results in an increased intracellular concentration of the metabolite. Optimal conditions for decreasing the Δp or one of its components will depend on the properties of the transport system involved. For instance, a decrease of the internal pH can drastically influence the activity of the transport system [68]. An important objection against decreasing the Δp is the possible detrimental effect on bacterial metabolism, and as a result a decreased metabolite production efficiency. Moreover, a decrease of Δp can result in a lowering of all intracellular metabolite pools which are transported by secondary transport systems.

In special cases, excretion of metabolites is catalysed by the same proton/solute transport system that mediates uptake of the precursor of the metabolic pathway. The substrate specificity of these systems permits the secretion of the metabolite (or endproduct of metabolism) as its chemical gradient exceeds Δp . Phenomenologically, these systems resemble the strictly coupled solute/solute antiport systems which are described in a later section. Examples of this group

of transport systems are lactose/galactose [78,79] and malate/lactate exchange (B. Poolman, D. Molenaar, E.J. Smid, T. Ubbink, T. Abee, P.P. Renault and W.N. Konings, submitted) in lactic acid bacteria and arginine/ornithine exchange in *Pseudomonas aeruginosa* [119] (Table 2). These systems are capable of heterologous exchange at rates that exceed the rate of solute uptake via the proton/solute symport mode.

5.3. Proton/solute antiport

Proton/solute antiport systems are optimally suited for the excretion of metabolites and end-products of metabolism for reasons that under physiological conditions the driving force for metabolite transport will be directed from inside to outside. As in the proton/solute symport systems the driving force is supplied by the electrochemical gradients of the protons and the excreted metabolite. Since both solutes move down their gradients the driving forces are cumulative (for example see eqn. 7.). Even in the absence of a metabolite gradient, the existing Δp will drive the metabolite out of the cell. Conditions may prevail in which the concentration of the metabo-

lite in the cell is lower than in the external medium.

Only a few proton/solute antiport systems are found in bacteria. H^+ -linked antiporters have been found for the secretion of K^+ , Na^+ , Ca^{2+} [80], and ethidium bromide [81,82]. Uptake of NH_4^+ in *E. coli* is mediated by a unique K^+/NH_4^+ ($CH_3NH_3^+$) antiport mechanism [83]. The antibiotic tetracycline is excreted by a wide variety of bacteria [84] by an electroneutral exchange with protons (or divalent cation-tetracycline complex/proton antiport [85]). Interestingly, these systems are closely related to the efflux systems for tetracycline [86] and methylenomycin A [87] in antibiotic-producing *Streptomyces* strains and the aminotriazole resistance determinant of *Saccharomyces cerevisiae* [88]. Lysine is excreted by a specific efflux transport system in *C. glutamicum* (S. Bröer and R. Krämer, personal communications). This excretion can proceed against a chemical gradient and is driven by Δp . Lysine is presumably excreted in a symport mechanism with two OH^- (energetically equivalent to an lysine/ $2H^+$ antiport mechanism). Excretion of glutamate by *C. glutamicum* is most likely ATP-

Table 2
Bacterial exchange systems

System ^a	Pathway	Organism [Reference]
Arginine/ornithine antiport	Arginine deiminase	<i>Lactococcus lactis</i> [102–106] <i>Streptococcus sanguis</i> , <i>Sc. milleri</i> , <i>Enterococcus</i> (<i>Ec.</i>) <i>faecalis</i> [106] <i>Pseudomonas aeruginosa</i> ^b
Arginine/ornithine exchange	Arginine deiminase	<i>Ec. faecalis</i> [107]
Agmatine/putrescine antiport	Agmatine deiminase	<i>Corynebacterium glutamicum</i> [108]
Lysine/alanine antiport ^c	Lysine uptake	<i>L. lactis</i> [109,110], <i>E. coli</i> [111], <i>Staphylococcus aureus</i> [112]
Glucose-6-phosphate/ P_i antiport	Glycolysis	
<i>sn</i> -glycerol 3-phosphate/ P_i antiport	Glycolysis	<i>E. coli</i> [113,114]
Phosphoenolpyruvate/ P_i antiport	Glycolysis	<i>Salmonella typhimurium</i> [93]
P_i / P_i antiport	?	<i>S. pyogenes</i> [115]
ATP/ADP antiport	ATP uptake	<i>Rickettsia prowazekii</i> [116,117]
Oxalate/formate antiport	Oxalate decarboxylation	<i>Oxalobacter formigenes</i> [118]
Malate/lactate exchange	Malate decarboxylation	<i>L. lactis</i> [119]
Lactose/galactose exchange	Glycolysis	<i>Sc. thermophilus</i> , <i>Lactobacillus bulgaricus</i> [78,79]

^a An antiporter mediates the strictly coupled exchange of substrates. Exchange signifies systems which catalyse an exchange reaction that is not strictly coupled. The group of proton-linked antiporter is not included in this list.

^b H. Verhoogt, H. Smid, D. Haas, A.J.M. Driessen and W.N. Konings, unpublished results.

^c Alanine can be replaced by isoleucine or valine as substrate.

driven [42,89]. Isoleucine is presumably secreted by a Δp -dependent system [90]. Efflux of 5'-inosinic acid [91] and NAD [46] from *Bv. ammoniagenes* are thought to result from a specific energy-dependent excretion process.

5.4. Solute / solute antiport

Many microorganisms metabolize their substrates (precursors) only partially and excrete the products of the metabolism into the medium. When precursors and products are structurally related, transport of both can be facilitated by the same transport protein. A prerequisite for precursor/product antiport is the stoichiometric conversion of precursor into end-product. The driving force for precursor/product antiport is composed of the chemical gradients of the precursor and product. The Δp contributes to the process when net movement of charge and/or protons take place. The linkage of precursor and product movements assures tight coupling to the corresponding metabolism. The different precursor/product antiport systems found in bacteria are summarized in Table 2 (For recent reviews see [31,68,78,80,92,93]).

6. ARE THERE WAYS TO OPTIMIZE SOLUTE TRANSPORT SYSTEMS FOR EXCRETION?

As discussed above, proton/product antiport is the mechanism of choice for excreting metabolites. The precursor/product antiport systems often are of limited use due to requirements for structural relatedness and stoichiometric conversion of the solutes. The question arises whether other transport systems, such as proton/solute symport systems, can be modified to proton/product antiport or uniport systems. In the latter case, the coupling of solute and proton movements has to be released. Another avenue is to change the specificity of known transport systems. Either approach requires detailed information about the function and structure of the transport systems. During recent years genes of many transport systems have been isolated and sequenced. On the basis of the hydropathy analysis, gene

fusions, immunological and biochemical studies, predictions have been made about the topology of proteins in the membrane. However, information about the three-dimensional structure of these proteins is not available as transport proteins have not yet been crystallized. Also mechanistic information on the actual transport reactions is scarce.

A well studied proton/solute symport system is the lactose carrier of *E. coli* [94]. The effects of an extensive number of site-specific amino acid substitutions on the functional properties of the transport protein have been examined. These studies suggest that three amino acids (catalytic triad), Arg-302, His-322 and Glu-325, present in two neighbouring membrane spanning loops, are involved in the translocation of the proton [94]. Until recently this was interpreted as their involvement only in H^+ movement. Mutants of the catalytic triad are now available which retained the ability to co-transport H^+ and lactose [95,96], and exhibit drastically lowered binding affinities for the sugar analogue nitrophenyl- α -D-galactoside [94]. An altered binding of the sugar molecule to the carrier may equally well explain the changed transport phenotype. Direct proof that the catalytic triad indeed constitutes the pathway of protons is not available. The major question of current energetics is related to the mechanism by which the movements of protons and lactose are coupled. One obvious conclusion which emerges from studies aimed to answer this question is that it will be very difficult, at least with our current knowledge, to reverse the pathway of the proton(s) thereby modifying a symport system into an antiport system. Another option is to change a proton/solute symport system into a solute (product) uniport system. Some mutants of the lactose carrier obtained by site-directed mutagenesis were found to catalyse lactose uniport [94]. Since in these mutants the movement of solute is not coupled to movement of protons, such mutants can be very attractive for mediating excretion processes.

Another form of protein-engineering which might be considered is to change the substrate (solute) specificity of available antiport systems by genetic manipulation. At this moment hardly

any information is available about the nature of the amino acid residues that determine substrate specificity. Random mutagenesis protocols in combination with direct selection have been used to isolate lactose carrier mutants with altered substrate specificity. In this way, mutants have been isolated that acquired the capacity to transport maltose while the capacity to transport lactose was diminished [97]. In case of the melibiose carrier of *E. coli*, mutants have been isolated with altered cation-ion or sugar specificity [98,99].

To improve amino acid secretion, another possible approach would be to make use of what 'nature' has to offer. The vacuolar membrane of *Saccharomyces cerevisiae* contains at least seven different proton/amino acid antiport systems [100,101]. These systems have a relatively low affinity for their substrate but a rather high specificity. It would be attractive to express the genes for these antiport systems in bacteria in order to enhance amino acid excretion. Another possibility is to use the bacterial excretion systems found thusfar to excrete solutes in other bacteria.

REFERENCES

- [1] Anraku, Y. (1988) Bacterial electron transfer chain. *Annu. Rev. Biochem.* 57, 101–132.
- [2] Prince, R.C. (1990) Bacterial photosynthesis: from photons to Δp . In: *The Bacteria* (T.A. Krulwich, Ed.), Vol. XII, pp. 111–149, Academic Press, New York, NY.
- [3] Unemoto, T., Tokuda, H. and Hayashi, M. (1990) Primary sodium pumps and their significance in bacterial energetics. In: *The Bacteria*, (T.A. Krulwich, Ed.), Vol. XII, pp. 33–54, Academic Press, New York, NY.
- [4] Khorana, H.G. (1988) Bacteriorhodopsin, a membrane protein that uses light to translocate protons. *J. Biol. Chem.* 263, 7439–7442.
- [5] Senior, A.E. (1988) ATP synthesis by oxidative phosphorylation. *Physiol. Rev.* 68, 177–231.
- [6] Laubinger, W. and Dimroth, P. (1987) Characterization of the Na^+ -stimulated ATPase of *Propionigenium modestum* as a enzyme of the F_1F_0 type. *Eur. J. Biochem.* 168, 475–480.
- [7] Epstein, W. (1990) Bacterial transport ATPases. In: *The Bacteria*, Vol. XII, (T.A. Krulwich, Ed.), pp. 87–110, Academic Press, New York, NY.
- [8] Tisa, L.S. and Rosen, B.P. (1990) Transport systems encoded by bacterial plasmids. *J. Bioenerg. Biomembr.* 22, 493–507.
- [9] Mergeay, M. (1991) Towards an understanding of the genetics of bacterial metal resistance. *Trends Biotechnol.* 9, 17–24.
- [10] Higgins, C.F., Hyde, S.C., Mimmack, M.M., Gileadi, U., Gill, D. R. and Gallagher, M.P. (1990) Binding protein-dependent transport systems. *J. Bioenerg. Biomembr.* 22, 571–592.
- [11] Alloing, G., Trombe, M.-C. and Claverys, J.-P. (1990) The *ami* locus of the Gram-positive bacterium *Streptococcus pneumoniae* is similar to binding protein-dependent transport operon of Gram-negative bacterium. *Mol. Microbiol.* 4, 633–644.
- [12] Dudler, R., Schmidhauser, C., Parish, R.W., Weptenhall, R.E.H. and Schmidt, T. (1988) A mycoplasma high-affinity transport system and the in vitro invasiveness of mouse sarcoma cells. *EMBO J.* 7, 3963–3970.
- [13] Perego, M., Higgins, C.F., Pearce, S.R., Gallagher, M.P. and Hoch, J.A. (1991) The oligopeptide transport system of *Bacillus subtilis* plays a role in the initiation of sporulation. *Mol. Microbiol.* 5, 173–185.
- [14] Kaback, H.R. (1988) Site-directed mutagenesis and ion-gradient driven active transport: On the path of the proton. *Annu. Rev. Physiol.* 50, 243–256.
- [15] Maloy, S.R. (1990) Sodium-coupled cotransport. In: *The Bacteria* (T.A. Krulwich, Ed.), Vol. XII, pp. 203–224, Academic Press, New York, NY.
- [16] Saier, M.H. Jr. and Chin, A.M. (1990) Energetics of the bacterial phosphotransferase system in sugar transport and the regulation of carbon metabolism. In: *The Bacteria* (T.A. Krulwich, Ed.), Vol. XII, pp. 273–299, Academic Press, New York, NY.
- [17] Kroll, J.S., Loynds, B., Brophy, L.N. and Moxon, E.R. (1990) The *bex* locus in encapsulated *Haemophilus influenzae*: a chromosomal region involved in capsule polysaccharide export. *Mol. Microbiol.* 4, 1853–1862.
- [18] Smith, A.N., Boulnois, G.J. and Roberts, I.S. (1990) Molecular analysis of the *Escherichia coli* K5 *kps* locus: identification and characterization of an inner-membrane capsular polysaccharide transport system. *Mol. Microbiol.* 4, 1863–1869.
- [19] Stanfield, S.W., Ielpi, L., O'Brochta, D., Helinski, D.R. and Ditta, G.S. (1988) The *ndvA* gene product of *Rhizobium meliloti* is required for β -(1 \rightarrow 2)glucan production and has homology to the ATP-binding export protein HlyB. *J. Bacteriol.* 170, 3522–3527.
- [20] Cangelosi, G.A., Martinetti, G., Leigh, J.A., Lee, C.C., Theines, C. and Nester, E.W. (1989) Role of *Agrobacterium tumefaciens* ChvA protein in export of β -1,2-Glucan. *J. Bacteriol.* 171, 1609–1615.
- [21] Ross, J.I., Eady, E.A., Cove, J.H., Cunliffe, W.J., Baumberg, S. and Wootton, J.C. (1990) Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. *Mol. Microbiol.* 4, 1207–1214.
- [22] Blight, M.A. and Holland, I.B. (1990) Structure and function of haemolysin B-P-glycoprotein and other

- members of a novel family of membrane translocators. *Mol. Microbiol.* 4, 873-880.
- [23] Reizer, J., Deutscher, J., Grenier, F., Thompson, J., Hengstenberg, W. and Saier, M.H.H. (1988) The phosphoenolpyruvate:sugar phosphotransferase system in gram-positive bacteria: Properties, mechanism, and regulation. *CRC Crit. Rev. Microbiol.* 15, 297-338.
- [24] Sten-Knudsen, O. (1978) Passive transport processes. In: *Membrane transport in Biology. I. Concepts and models* (G. Giebish, D.C. Tosteson and H.H. Using, Eds.), pp. 5-112. Springer Verlag, Berlin.
- [25] Stein, W.D. (1986) Transport and diffusion across cell membranes. Academic Press, Orlando, FL.
- [26] Rottenberg, H. (1975) The measurement of transmembrane electrochemical proton gradients. *J. Bioenerg.* 7, 61-74.
- [27] Walter, A. and Gutknecht, J. (1984) Monocarboxylic acid permeation through lipid bilayer membranes. *J. Membr. Biol.* 77, 255-264.
- [28] Ingram, L.O. (1976) Adaptation of membrane lipids to alcohols. *J. Bacteriol.* 125, 670-678.
- [29] Ingram, L.O. and Buttk, T.M. (1984) Effects of alcohols on microorganisms. *Adv. Microb. Physiol.* 25, 253-300.
- [30] Vollherbst-Schneck, K., Sands, J.A. and Montencourt, B.S. (1984) Effect of butanol on lipid composition and fluidity of *Clostridium acetobutylicum* ATCC 824. *Appl. Environ. Microbiol.* 47, 193-194.
- [31] Driessen, A.J.M. and Konings, W.N. (1990) Energetic problems of bacterial fermentations: Extrusion of metabolic end products. In: *The Bacteria* (T.A. Krulwich, Ed.), Vol. XII, pp. 449-478, Academic Press, New York, NY.
- [32] Kell, D.B., Peck, M.W., Rodger, G. and Morris, J.G. (1981) On the permeability of weak acids and bases of the cytoplasmic membrane of *Clostridium pasteurianum*. *Biochem. Biophys. Res. Commun.* 99, 81-88.
- [33] Baronofsky, J.J., Schreurs, W.J.A. and Kashket, E.R. (1984) Uncoupling by acetic acid limits growth of and acetogenesis by *Clostridium thermoaceticum*. *Appl. Environ. Microbiol.* 48, 1134-1139.
- [34] Monot, F., Engasser, J.M. and Petitdemange, H. (1984) Influence of pH and undissociated butyric acid on the production of acetone and butanol in batch cultures of *Clostridium acetobutylicum*. *Appl. Environ. Biotechnol.* 19, 422-426.
- [35] Awang, G.M., Jones, G.A. and Ingledew, W.M. (1988) The acetone-butanol-ethanol fermentation. *CRC Crit. Rev. Microb.* 15, S33-S67.
- [36] Gottwald, M. and Gottschalk, G. (1986) The internal pH of *Clostridium acetobutylicum* and its effect on the shift from acid to solvent formation. *Arch. Microbiol.* 143, 42-46.
- [37] Demain, A.L. and Birnbaum, J. (1968) Alteration of permeability for the release of metabolites from microbial cells. *Curr. Top. Microbiol.* 46, 1-25.
- [38] Clement, Y., Escoffier, B., Trombe, M.C. and Lanéele, G. (1984) Is glutamate excreted by its uptake system in *Corynebacterium glutamicum*? A working hypothesis. *J. Gen. Microbiol.* 130, 2589-2594.
- [39] Clement, Y. and Lanéele, G. (1986) Glutamate excretion mechanism in *Corynebacterium glutamicum*: triggering by biotin starvation or by surfactant addition. *J. Gen. Microbiol.* 132, 925-929.
- [40] Luntz, M.G., Zhdanova, N.I. and Bourd, G.I. (1986) Transport and excretion of L-lysine in *Corynebacterium glutamicum*. *J. Gen. Microbiol.* 132, 2137-2146.
- [41] Shio, I., Otsuka, S.I. and Takahashi, M. (1962) Effect of biotin on the bacterial formation of glutamic acid. I. Glutamate formation and cellular permeability of amino acids. *J. Biochem.* 51, 56-62.
- [42] Hoischen, C. and Krämer, R. (1989) Evidence for an efflux carrier involved in the secretion of glutamate by *Corynebacterium glutamicum*. *Arch. Microbiol.* 151, 342-347.
- [43] Bunch, A.W. and Harris, R.E. (1986) The manipulation of microorganisms for the production of secondary metabolites. *Biotechnol. Gen. Engin. Rev.* 4, 117-144.
- [44] Nakao, Y., Kikuchi, M., Suzuki, M. and Doi, M. (1972) Microbial production of L-glutamic acid by glycerol auxotrophs. *Agric. Biol. Chem.* 36, 490-496.
- [45] Teshiba, S. and Furuya, A. (1983) Mechanisms of 5'-inosinic acid accumulation by permeability mutants of *Brevibacterium ammoniagenes*. III. Intracellular 5'-IMP pool and excretion mechanisms of 5'-IMP. *Agric. Biol. Chem.* 47, 2357-2363.
- [46] Schmid, J. and Auling, G. (1989) Alterations of the membrane composition induced by manganese depletion are late events in the nucleotide fermentation with *Brevibacterium ammoniagenes* ATCC 6872. *Agric. Biol. Chem.* 53, 1783-1788.
- [47] Driessen, A.J.M., Hellingwerf, K.J. and Konings, W.N. (1987) Mechanism of energy coupling to entry and exit of neutral and branched chain amino acids in membrane vesicles of *Streptococcus cremoris*. *J. Biol. Chem.* 262, 12438-12443.
- [48] Driessen, A.J.M., de Vrij, W. and Konings, W.N. (1985) Incorporation of beef heart cytochrome c oxidase as a proton-motive force-generating mechanism in bacterial membrane vesicles. *Proc. Natl. Acad. Sci. USA* 82, 7555-7559.
- [49] Nozaki, Y. and Tanford, C. (1971) The solubility of amino acids and two glycine peptides in aqueous ethanol and dioxane solutions. Establishment of a hydrophobicity scale. *J. Biol. Chem.* 246, 2211-2217.
- [50] Klein, R.A., Moore, M.J. and Smith, M.W. (1971) Selective diffusion of neutral amino acids across lipid bilayers. *Biochim. Biophys. Acta* 233, 430-433.
- [51] Wilson, P.D. and Wheeler, K.P. (1973) Permeability of phospholipid vesicles to amino acids. *Biochem. Soc. Trans. London* 1, 369-372.
- [52] Smid, E.J. and Konings, W.N. (1989) Relationship be-

- tween utilization of proline and proline-containing peptides and growth of *Lactococcus lactis*. *J. Bacteriol.* 172, 5286–5292.
- [53] Rosen, B.P. (1973) Basic amino acid transport in *Escherichia coli*: properties of canavanine-resistant mutants. *J. Bacteriol.* 116, 627–635.
- [54] Halsall, D.M. (1975) Overproduction of lysine by mutant strains of *Escherichia coli* with defective lysine transport. *Biochem. Genet.* 13, 109–124.
- [55] Anderson, J.J. and Oxender, D.L. (1978) Genetic separation of high- and low-affinity transport systems for branched-chain amino acids in *Escherichia coli* K-12. *J. Bacteriol.* 136, 168–174.
- [56] Rancourt, D.E., Stephenson, J.T., Vickell, G.A. and Wood, J.M. (1984) Proline excretion by *Escherichia coli* K-12. *Biotechnol. Bioenerg.* 26, 74–80.
- [57] Shifrin, S., Ames, B.N. and Ames, G.F.L. (1966) Effect of the hydrazino analogue of histidine on histidine transport and arginine biosynthesis. *J. Biol. Chem.* 241, 3424–3429.
- [58] Anderson, R.R., Menzel, R. and Wood, J.M. (1980) Biochemistry and regulation of a second L-proline transport system in *Salmonella typhimurium*. *J. Bacteriol.* 141, 1071–1076.
- [59] Hongo, M., Gan, B.H. and Uyeda, M. (1974) Extracellular formation of alanine by anaerobes. *Agr. Biol. Chem.* 38, 1805–1810.
- [60] Matteuzzi, D., Crociani, F. and Emaldi, O. (1978) Amino acid production by Bifidobacteria and some Clostridia. *Ann. Microbiol. (Inst. Pasteur)* 129B, 175–181.
- [61] Szech, U., Braun, M. and Kleiner, D. (1989) Uptake and excretion of amino acids by saccharolytic clostridia. *FEMS Microbiol. Lett.* 58, 11–14.
- [62] Ten Brink, B. and Konings, W.N. (1980) Generation of an electrochemical proton gradient by lactate efflux in membrane vesicles of *Escherichia coli*. *Eur. J. Biochem.* 111, 59–66.
- [63] Otto, R., Sonnenberg, A.S.M., Veldkamp, H. and Konings, W.N. (1980) Generation of an electrochemical proton gradient in *Streptococcus cremoris* by lactate efflux. *Proc. Natl. Acad. Sci. USA* 77, 5502–5506.
- [64] Ten Brink, B. and Konings, W.N. (1982) Electrochemical proton gradient and lactate concentration gradient in *Streptococcus cremoris* cells grown in batch culture. *J. Bacteriol.* 152, 682–686.
- [65] Ten Brink, B., Otto, R., Hansen, U.P. and Konings, W.N. (1985) Energy recycling by lactate efflux in growing and nongrowing cells of *Streptococcus cremoris*. *J. Bacteriol.* 162, 383–390.
- [66] Otto, R., Lageveen, R.G., Veldkamp, H. and Konings, W.N. (1982) Lactate efflux-induced electrical potential in membrane vesicles of *Streptococcus cremoris*. *J. Bacteriol.* 149, 733–738.
- [67] Konings, W.N. and Booth, I.R. (1981) Do the stoichiometries of ion-linked transport systems vary? *Trends Biochem. Sci.* 6, 257–262.
- [68] Poolman, B., Driessen, A.J.M. and Konings, W.N. (1987) Regulation of solute transport in Streptococci by the external and internal pH values. *Microbiol. Rev.* 51, 498–508.
- [69] Michels, P.A.M., Michels, J.P.J., Boonstra, J. and Konings, W.N. (1979) Generation of an electrochemical gradient in bacteria by excretion of metabolic end products. *FEMS Microbiol. Lett.* 5, 357–364.
- [70] Simpson, S.J., Bendall, M.R., Egan, A.F. and Rogers, P.J. (1983) High field phosphorus NMR studies of the stoichiometry of the lactate/proton carrier in *Streptococcus faecalis*. *Eur. J. Biochem.* 136, 63–69.
- [71] Simpson, S.J., Vink, R., Egan, A.F. and Rogers, P.J. (1983) Lactate efflux stimulate (^{32}P) ATP exchange in *Streptococcus faecalis* membrane vesicles. *FEMS Microbiol. Lett.* 5, 85–88.
- [72] Michel, T.A. and Macy, J.M. (1990) Generation of a membrane potential by sodium-dependent succinate efflux in *Selenomonas ruminantium*. *J. Bacteriol.* 172, 1430–1435.
- [73] McInerney, M.J. and Beaty, P.S. (1988) Anaerobic community structure from a nonequilibrium thermodynamic perspective. *Can. J. Microbiol.* 34, 487–493.
- [74] Ingvorsen, K., Zehnder, A.J.B. and Jørgensen, B.B. (1984) Kinetics of sulfate and acetate uptake by *Desulfohalobacter postgatei*. *Appl. Environ. Microbiol.* 47, 404–408.
- [75] Schönheit, P., Kristjansson, J.K. and Thauer, R.K. (1982) Kinetic mechanism for the ability of sulfate reducers to outcompete methanogens for acetate. *Arch. Microbiol.* 132, 285–288.
- [76] Boenigk, R., Dürre, P. and Gottschalk, G. (1989) Carrier-mediated acetate transport in *Acetobacterium woodii*. *Arch. Microbiol.* 152, 589–593.
- [77] Smith, M.R. and Lequerica, J.L. (1985) *Methanosarcina* mutant unable to produce methane or assimilate carbon from acetate. *J. Bacteriol.* 164, 618–625.
- [78] Poolman, B. (1990) Precursor/product antiport in bacteria. *Mol. Microbiol.* 4, 1629–1636.
- [79] Hutkins, R.W. and Ponne, C. (1991) Lactose uptake driven by galactose efflux in *Streptococcus thermophilus*: evidence for a galactose–lactose antiport. *Appl. Environ. Microbiol.* 57, 941–944.
- [80] Ambudkar, S.V. and Rosen, B. (1990) Ion-exchange systems in prokaryotes. In: *The Bacteria* (T.A. Krulwich, Ed.), Vol. XII, pp. 247–271, Academic Press, New York, NY.
- [81] Purewal, A.S., Jones, I.G. and Midley, M. (1990) Cloning of the ethidium gene from *Escherichia coli*. *FEMS Microbiol. Lett.* 68, 73–76.
- [82] Neyfakh, A.A., Bidnenko, V.E. and Chen, L.B. (1991) Efflux-mediated multidrug resistance in *Bacillus subtilis*: Similarities and dissimilarities with the mammalian system. *Proc. Natl. Acad. Sci. USA* 88, 4781–4785.
- [83] Jayakumar, A., Epstein, W. and Barnes, E.M. Jr. (1985) Characterization of ammonium (methylammonium)/potassium antiport in *Escherichia coli*. *J. Bacteriol.* 260, 7528–7532.

- [84] Levy, S.B. (1989) Evolution and spread of tetracycline resistance determinants. *J. Antimicrob. Chemother.* 24, 1-3.
- [85] Yamaguchi, A., Udagawa, T. and Sawai, T. (1990) Transport of divalent cations with tetracycline as mediated by transposon Tn10-encoded tetracycline resistance protein. *J. Biol. Chem.* 265, 4809-4813.
- [86] Cunliffe, E. (1989) How antibiotic-producing organisms avoid suicide. *Annu. Rev. Microbiol.* 43, 207-233.
- [87] Neal, R.J. and Chater, K.F. (1987) Nucleotide sequence analysis reveals similarities between proteins determining methylenomycin A resistance in *Streptomyces* and tetracycline resistance in eubacteria. *Gene* 58, 229-241.
- [88] Kanazawa, S., Driscoll, M. and Struhl, K. (1988) ATR1, a *Saccharomyces cerevisiae* gene encoding a transmembrane protein required for aminotriazole resistance. *Mol. Cellular Biol.* 8, 664-673.
- [89] Hoischen, C. and Krämer, R. (1990) Membrane alteration is necessary but not sufficient for effective glutamate secretion in *Corynebacterium glutamicum*. *J. Bacteriol.* 172, 3409-3416.
- [90] Ebbighausen, H., Weil, B. and Krämer, R. (1989) Isoleucine excretion in *Corynebacterium glutamicum*: evidence for a specific efflux carrier system. *Appl. Microbiol. Biotechnol.* 31, 184-190.
- [91] Teshiba, S. and Furuya, A. (1984) Mechanisms of 5'-inosinic acid accumulation by permeability mutants of *Brevibacterium ammoniagenes*. IV. Excretion mechanisms of 5'-IMP. *Agric. Biol. Chem.* 48, 1311-1317.
- [92] Maloney, P.C., Ambudkar, S.V., Anantharam, V., Sonna, L.A. and Varadhachary, A. (1990) Anion exchange mechanisms in bacteria. *Microbiol. Rev.* 54, 1-17.
- [93] Maloney, P.C. (1990) Anion exchange reactions in bacteria. *J. Bioenerg. Biomembr.* 22, 509-523.
- [94] Kaback, H.R. (1990) Active transport: membrane vesicles, bioenergetics, molecules and mechanisms. In: *The Bacteria* (T.A. Krulwich, Ed.), Vol. XII, pp. 151-202, Academic Press, New York, NY.
- [95] King, S.C. and Wilson, T.H. (1989) Galactoside-dependent proton transport by mutants of the *Escherichia coli* lactose carrier. *J. Biol. Chem.* 264, 7390-7394.
- [96] Brooker, R.J. (1990) Characterization of the double mutant, Val-177/Asn-322, of the lactose permease. *J. Biol. Chem.* 265, 4155-4160.
- [97] Brooker, R.J. and Wilson, T.H. (1985) Isolation and nucleotide sequencing of lactose carrier mutants that translocate maltose. *Proc. Natl. Acad. Sci. USA* 82, 3959-3963.
- [98] Yazuy, H., Shiota, S., Futai, M. and Tsuchiya, T. (1985) Alteration in cation specificity of the melibiose transport carrier of *Escherichia coli* due to replacement of proline 122 with serine. *J. Bacteriol.* 162, 933-937.
- [99] Botfield, M.C. and Wilson, T.H. (1988) Mutations that simultaneously alter both sugar and cation specificity in the melibiose carrier of *Escherichia coli*. *J. Biol. Chem.* 263, 12909-12915.
- [100] Sato, T., Ohsumi, Y. and Anraku, Y. (1984) Substrate specificities of active transport systems for amino acids in vacuolar-membrane vesicles of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 259, 11505-11508.
- [101] Sato, T., Ohsumi, Y. and Anraku, Y. (1984) An arginine/histidine exchange transport system in vacuolar-membrane vesicles of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 259, 11509-11511.
- [102] Driessen, A.J.M., Poolman, B., Kiewiet, R. and Konings, W.N. (1987) Arginine transport in *Streptococcus lactis* is catalysed by a cationic exchanger. *Proc. Natl. Acad. Sci. USA* 84, 6093-6097.
- [103] Driessen, A.J.M., van Leeuwen, R. and Konings, W.N. (1989) Transport of basic amino acids by membrane vesicles of *Lactococcus lactis*. *J. Bacteriol.* 171, 1453-1458.
- [104] Driessen, A.J.M., Molenaar, D. and Konings, W.N. (1989) Kinetics and regulation of arginine:ornithine exchange in membrane vesicles of *Lactococcus lactis*. *J. Biol. Chem.* 264, 10361-10370.
- [105] Thompson, J. (1987) Ornithine transport and exchange in *Streptococcus lactis*. *J. Bacteriol.* 169, 4147-4153.
- [106] Poolman, B., Driessen, A.J.M. and Konings, W.N. (1987) Regulation of arginine-ornithine exchange and the arginine deiminase pathway in *Streptococcus lactis*. *J. Bacteriol.* 169, 5597-5604.
- [107] Driessen, A.J.M., Smid, E. and Konings, W.N. (1988) Transport of diamines by *Enterococcus faecalis* is mediated by an agmatine-putrescine antiporter. *J. Bacteriol.* 170, 4522-4527.
- [108] Bröer, S. and Krämer, R. (1990) Lysine uptake and exchange in *Corynebacterium glutamicum*. *J. Bacteriol.* 172, 7241-7248.
- [109] Maloney, P.C., Ambudkar, S.V., Thomas, J. and Schiller, L. (1984) Phosphate/hexose 6-phosphate antiport in *Streptococcus lactis*. *J. Bacteriol.* 158, 238-245.
- [110] Ambudkar, S.V., Sonna, L.A. and Maloney, P.C. (1986) Variable stoichiometry of phosphate-linked anion exchange in *Streptococcus lactis*: Implications for the mechanism of sugar phosphate transport by bacteria. *Proc. Natl. Acad. Sci. USA* 83, 280-284.
- [111] Sonna, L.A., Ambudkar, S.V. and Maloney, P.C. (1988) The mechanism of glucose 6-phosphate transport by *Escherichia coli*. *J. Biol. Chem.* 263, 6625-6630.
- [112] Sonna, L.A. and Maloney, P.C. (1988) Identification and functional reconstitution of phosphate:sugar phosphate antiport of *Staphylococcus aureus*. *J. Membr. Biol.* 101, 267-274.
- [113] Elvin, C.M., Hardy, C.M. and Rosenberg, H. (1985) P_i exchange mediated by the GlpT-dependent sn-glycerol-3-phosphate transport system in *Escherichia coli*. *J. Bacteriol.* 161, 1015-1058.
- [114] Eigelmeier, K., Boos, W. and Cole, S.T. (1987) Nu-

- cleotide sequence and transcriptional start point of the *glpT* gene of *Escherichia coli*: extensive sequence homology of the glycerol-3-phosphate transport system with components of the hexose-6-phosphate transport system. *Mol. Microbiol.* 1, 251–258.
- [115] Reizer, J. and Saier, M.H. Jr. (1987) Mechanism and regulation of phosphate transport in *Streptococcus pyrogenes*. *J. Bacteriol.* 169, 297–301.
- [116] Winkler, H.H. (1976) Rickettsial permeability. An ADP–ATP transport system. *J. Biol. Chem.* 251, 389–396.
- [117] Krause, D.C., Winkler, H.H. and Wood, D.O. (1985) Cloning and expression of the *Rickettsia prowazekii* ATP/ADP translocator in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 82, 3015–3019.
- [118] Anantharam, V., Allison, M.J. and Maloney, P.C. (1989) Electrogenic oxalate:formate exchange, the basis of energy coupling in *Oxalobacter formigenes*. *J. Biol. Chem.* 264, 7244–7250.
- [119] Poolman, B., Molenaar, D., Smid, E.J., Ubbink, T., Abee, T., Renault, P.P. and Konings, W.N. (1991) Malolactic fermentation: electrogenic malate uptake and malate/lactate antiport generate metabolic energy. *J. Bacteriol.* 173, 6030–6037.